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Extraction of bioactive compound from *Acacia seyal* gum, *in vitro* evaluation of antitumor activity of its crude extract against leukemia

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ABSTRACT

Today, many therapy drugs have been used to treat cancer patients. However, those drugs are not effective enough and usually have adverse side effects on human health. Different herbal medicine is rising in popularity because it is more compatible with the human body and has fewer side effects. Even while alternative herbal remedies effectively decrease symptoms in traditional medicine, many of them have yet to be scientifically proven. As a result, it's critical to keep looking for ways to recover its efficiency against cancer cells. *Acacia seyal* gum (ASG), known as Arabic gum, is a well-known traditional medicinal therapy with various restorative characteristics. In this study, the yield of ASG extract was optimized using experimental design followed by chemical characterization of a bioactive compound for the last yield, then the therapeutic potential of ASG crude extracts against leukemia cancer cells was investigated *in vitro*. The Raman Spectroscopy (RS), Fourier Transform Infrared (FTIR) Spectroscopy, and GC-TOFMS analyses were used to characterize ASG crude hydroethanolic extract bioactive components. The anti-leukemic activity of ASG crude extracts was investigated *in vitro* against tumoral Jurkat T-cell ALL, and K562 leukemia cancer cell lines, as well as nontumoral WIL2NS cells. The optimum extraction conditions resulted in a yield of 75.89% after 45 min of extraction at temperature 40 °C and solid/liquid ratio of 1:25 g/ml. The cytotoxicity assays of ASG and Taxol revealed that both treatments inhibited the growth of K562 and Jurkat T cancer cells and exhibited the lowest IC_{50} for K562 and Jurkat T cancer cell (IC_{50}=10 g/ml and IC_{50}=5.11 g/ml, respectively), and a negligible inhibition effect for WIL2NS cells (IC_{50}=80 g/ml).

1. Introduction

Leukemia is a cancerous tissue-forming disease that triggers the excessive production of immature blood cells entering the bloodstream (Azher and Shiggaon, 2013). Leukemia cell proliferation occurs primarily in the bone marrow, with several forms in the lymphoid tissue (Naumburg et al., 2002). Leukemia has been recognized since the publication of reports of patients who died of the disease in 1845 and had elevated blood cells (Naumburg et al., 2002). According to the World Health Organization (WHO), cancer deaths will increase by 104% worldwide by 2020 (Dutta et al., 2019).

Currently, leukemia is among the most prevalent types of cancer in various parts of the world. In the US, leukemia may cause mortality of an estimated 58.100 people in 2018, and the type of cancer suffered among adolescents and young adults below 20 years in the same country is leukemia (Collins, 1987). In Malaysia, the incidence of lymphocytic leukemia in both men and women was 2.8 and 1.7 per 100.000 population, respectively, while the incidence of myeloid leukemia in both men and women was per 100.000, respectively, it was 3.0 and 2.7 (Saedi et al., 2014).

Patients with leukemia are treated with combination therapies such as chemotherapy (primary treatment), antibiotics, blood transfusions, radiation therapy, and bone marrow transplantation. While these treatments have helped increase the survival rate of leukemia patients, some of these treatments are difficult to practice. Thus, there is a need to look for alternative remedies to cure leukemia. Therefore, the use of natural products has increased

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gradually to discover a new antitumor drug that has few side effects on the immune system and is an important target in many immunopharmacological studies (Xu et al., 2017).

Hence, these natural plant products should be studied to understand their properties, protection, and effectiveness. It would result in reduced use of most common therapies for cancer such as radiation therapy, surgery, and chemotherapy. Some cancer cells showed resistance to chemotherapy treatment; as a researcher, we need to discover new cytotoxic drugs that function through distinctive mechanisms.

In developing countries, using therapeutic herbs as cures for leukemia is gaining popularity. About 35.000 plant samples were collected by National Cancer Institute from 20 countries, and around 114.000 extracts were screened for anticancer activity (Prakash et al., 2013).

Plants are known to be one of the most important sources of bioactive substances. Various modules of studies showed that constituents of plants demonstrated various biological and pharmacological activities. For thousands of years, plants were utilized to treat various diseases. Different plant part like leaves, barks, fruits, leaves due to their bioactive compounds acted as anticancer following different mechanism of action like an inhibitor of carcinogen formation, blockers of carcinogen interaction, and suppressor of tumor progression according to the chemical compound in the plant (Saedi et al., 2014).

In Australia, northern tropical Africa, and Egypt (Awad et al., 2018), *Acacia seyal* gum (ASG) is popular due to its medicinal use and is known as the second most important source of Arabic gum product (Nie et al., 2013) (Figure 1) after *Acacia senegal*. Numerous studies showed that constituents of ASG have various biological and pharmacological activities where it has a lot of dietary fibers and polyphenolic compounds, which can improve human health due to its antioxidant activity (Elnour et al., 2018b). It was used in traditional medicine as an astringent against colds, diarrhea, hemorrhage, ophthalmia, bronchitis, rheumatism, leprosy, also used against cancer due to its powerful anticarcinogenic effect on specific cancer cell lines (Ahmed, 2018). Much research has been found that ASG has been found in many applications in medicine and healthcare due to the increased quantities of alkaloids, flavonoids, glycosides, and saponins found in the ASG. The traditional practice and medical application of using bark juice to cure wounds were observed (Mbaveng et al., 2014; Suriyamoorthy et al., 2014). The extracts of ASG have been reported to have other different pharmacologic effects like antipyretic, anti-inflammatory, anti-diarrheal, hypoglycemic, hepatoprotective, antioxidant, and antimicrobial activities.

Figure 1. *Acacia seyal* gum powder

Recently, consumption of ASG increased following the discovery of phytochemical constituents such as phenolic compounds and flavonoids that are associated with a variety of pharmacological activities (Elmi et al., 2020). At present, more attention is being focused on the anticancer properties of ASG, and they tested its cytotoxicity against various kinds of cancer cell lines.

Acacia seyal (Del.) tree belongs to the family Mimosaceae of the genus *Acacia* and is usually distributed in tropical Africa, mainly found in West Africa, East Africa, and the Arabian Peninsula (Awad et al., 2018). *A. seyal* tree grows up to 17 m tall and 60 cm in diameter, with a flat top crown. Like most Acacias, it grows vigorously, coppice readily, and withstand heavy browsing. The leafy branches can be cut for fodder within the growing season without significant damage to tree performance (El Mahi and Magid, 2014). It has a characteristic smooth powdery rind that varies from white to greenish-yellow or orange-red, with a layer of green beneath it. Some populations have both red and yellow bark trees. The gum is used for biotechnology research and the food industry because of its clarity and solubility. The bark leaves and gums of the *A. seyal* tree are used for many medicinal purposes, for example, as a pharmaceutical ingredient in medication for throat and stomach inflammation and as a film-forming agent in peel-off skin masks, colds, hemorrhage, jaundice, headache, burns and chronic renal failure (Glover, 2012). However, exposure to smoke is believed to relieve rheumatic pains; the bark is used against leprosy, skin lesions, and dysentery, is a stimulant, and acts as a laxative for humans and animals.

Various chemical compounds have been isolated and characterized from ASG like flavonoids, particularly flavonols quercetin which bark showed cytotoxic activity *in vitro* (Murakami et al., 2008). ASG has been primarily composed of complex polysaccharides and contains a small amount of protein. Traditional African system medicine is used to treat many illnesses such as infertility, skin diseases, and cancer.

2. Materials and methods

Extraction using the Ultrasound-Assisted Extraction (UAE) method of the bioactive compounds from ASG and optimizing the condition of extraction to get high yield to depend on a Face-Centred Central Composited Design (FCCCD) under Response Surface Methodology (RSM) following the method of Fan et al.(2020). Next, the qualitative identification of the most bioactive compounds available in ASG was carried out using Raman Spectroscopy, FTIR Spectroscopy, and GC-TOFMS analysis. Finally, the crude extract was tested *in vitro* for antiproliferative effects against WIL2NS, K562, and Jurkat -T human leukemia cell lines using the MTT assay, with Taxol as a positive control.

2.1. Sample preparation, gum extraction

ASG nodules were taken from Blue Nile State, Sudan. The samples were cleaned from impurities such as bark and sand. For homogeneity of the sample, the ASG nodule was selected randomly from the other nodules. Then it was ground to powder and sieved by U.S.A standard testing sieve (Fisher Scientific, Massachusetts, USA) with a 1.40 mm mesh size. The finished ASG powder was packed and stored in a polyethylene ziplock bag at 4 °C until further analysis. Sampling was performed only one time.

The bioactive metabolite extraction from plant materials was performed according to the method of Adwan et al. (2010) and Esmaeili et al. (2021) with some adjustments. The optimum extraction parameters were systematically examined in this study. The gum powder samples (3 g) were mixed with 30 ml of 60% ethanol (v/v) (ethanol was diluted with distilled water), *n*-hexane, and acetone mixed in a 200 ml beaker for OFAT experimental design (Table 1), then covered with aluminum foil and sonicate using 40 kHz, power level 5, at room temperature as a standard parameter. The presence of different organic solvents was attempted to assess their capability to get the best yield of extract from ASG solvents, including acetone, 60% ethanol, and hexane. The extraction process followed the standard protocol.

2.2. Ultrasound-Assisted Extraction (UAE) optimization

The experimental design was intended to study and observe the effects of the extraction protocol, extraction procedure on ASG extraction yield (%), and the content of bioactive compounds obtained. During the preliminary test, it was found that in addition to the type of extraction solvent, several factors such as extraction temperature, time, and solid-liquid ratio influence ASG extraction (Norshazila et al., 2017). All experiments were performed 3 times. Extraction yield (%) was defined as reaction (Y).

Table 1. Experimental design and levels of independent process variables

Symbol	Independent variables	Low level	High level
A	Extraction time (min)	30.00	60.00
B	Extraction temp (°C)	30.00	50.00
C	Solid-liquid ratio (gm/l)	15.00	25.00

2.3. Statistical optimization of experiments

The design of the experiment has been applied to improve the quality of the extraction processes and the extract yield. Additionally, to make these products healthy to extreme conditions. The application of statistical optimization experiments using various factors related to the manufacturing process is well documented. The RSM is employed to significantly improve extraction processes and the extract (Pandey et al., 2018). These procedures were performed for analyzing different factors at once. We also reduced the number of related experiments, improved the interpretation of the data, and reduced the time required for the experiments. In this study, Expert Design Software v.12.0.0 (Stat-Ease Inc., USA) proved remarkably useful in establishing the optimal ASG extraction parameters that optimize the extraction yield.

A traditional One-Factor Data Time (OFAT) test design was used to determine the optimal solvent for maximizing the yield of ASG extract. The earlier literature showed that the solvent used for extraction affects extraction yield, phytochemical contents, and other factors like extraction time, temperature, and solid-liquid ratio (Abolmaesoomi et al., 2019; Che Sulaiman et al., 2017).

The design of experiment findings, which was determined from the FCCCD, contains 20 runs of experiments; each experiment was performed 3 times. Answer (Y) was the percentage of extraction yield. Analysis of variance (ANOVA) was evaluated by performing statistical analysis of the model. The relationship between the response (dependent factor) and the experimental level of each variable in this study was described from the viewpoint of contour lines and response surface graph, and an approximate polynomial was confirmed.

2.4. Preparation of solutions and reagents

Due to the possibility of contamination from reagents and materials, all apparatus was sterilized at 121 °C and 15 psi for 15-20 minutes using the autoclave method. This effect was important because the cell culture was naturally insensitive to contamination. Cell lines were maintained in complete cell culture or growth medium RPMI 1640 (Sigma Aldrich, USA). The medium was supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The cells were cultured at 37 °C. under 5% CO_2 conditions in a cell culture laboratory at IIUM's Department of Biotechnology Engineering, Malaysia. The culture medium with 10 % (v/v) FBS was freshly prepared to ensure sterility and extend the medium's shelf life. Firstly, FBS was filtered inside the biosafety cabinet using a 0.45 µm filter to ensure sterility. Then 100 ml of FBS (GIBCO, USA) was added to 900 ml of cell culture medium, and the solution was mixed gently to avoid the formation of a bubble. The remaining liquid medium was placed inside the chiller at 4 °C. Each time this medium was used, the temperature changed to 37 °C in a water bath.

3. Results and discussion

3.1. Optimisation of solvent using OFAT

There is limited study on the effect of polar solvent in ultrasound extraction on the yield and the bioactive compound of ASG crude extract. In contrast, a survey conducted by (Elnour et al., 2020) reported that using methanol as solvent of extraction explained that the maximum yield of extract was 11.10% - 15.56% for ASG. While ethanol is more desirable because it is a commercial solvent widely used for the extraction process, it is non-toxic, has a suitable polarity, and can dissolve bioactive compounds. It has been suggested that ASG could be more soluble in methanol and ethanol than other organic solvents. Therefore, Elnour et al. (2018a) explored that methanol for extraction of raw ASG was found to be more effective in all the assays than the chloroform, hexane, acetone fractions. Lower extraction yield when using acetone and hexane compared to 60% ethanol could be due to the low solubility of gum in acetone and hexane. In contrast, ethanol can dissolve both polar and non-polar substances. The polar character of ethanol is higher than acetone.

3.2. Significance of the regression

The regression coefficient R^2 values demonstrated the effect of each variable on the experimental response, while the predicted R^2 (0.89) was in reasonable agreement with the adjusted R^2 (0.96) (Table 2). The higher values of R^2 (0.90) and the closest to the adjusted R^2 (0.96) for extraction yield also revealed the model's efficacy. The Lack of Fit F-value of 0.38 indicates that the Lack of Fit is insignificant ($p > 0.05$) in comparison to the pure error for extraction yield. Thus, they proved that the model fits the data and demonstrated its apparent adequacy (Table 2). In addition, the answer is that the linear coefficients time (A) and temperature (B) and quadratic coefficients (A, B, A2, and B2) are ($p < 0.05$), and since the extraction yield, an important model term for this study. It shows that it is. A minimal *p*-value ($p < 0.05$) indicates that extraction time and temperature correlate with extraction yield.

Table 2. ANOVA for yield (%) fitted quadratic model of extraction conditions

Source	Sum of squares	Degree of freedom	Mean square	F-value	*p*-value	
Model	431.23	9	47.91	53.20	< 0.0001	significant
A-Time	0.5429	1	0.5429	0.6028	0.0045	
B-Temp	3.21	1	3.21	3.57	0.0088	
C-S/L	3.59	1	3.59	3.98	0.0739	
AB	2.73	1	2.73	3.03	0.1125	
AC	0.2211	1	0.2211	0.2455	0.6310	
BC	0.0561	1	0.0561	0.0623	0.8079	
A^2	99.96	1	99.96	111.00	< 0.0001	
B^2	44.64	1	44.64	49.57	< 0.0001	
C^2	0.1090	1	0.1090	0.1210	0.7351	
Residual	9.01	10	0.9006			
Lack of fit	5.14	5	1.03	1.33	0.3808	not significant
Pure error	3.86	5	0.7728			
Cor total	440.24	19				

Model terms with *p*-values < 0.05 were significant. The *p*-value of 0.38 indicated that the lack of fit was insignificant.

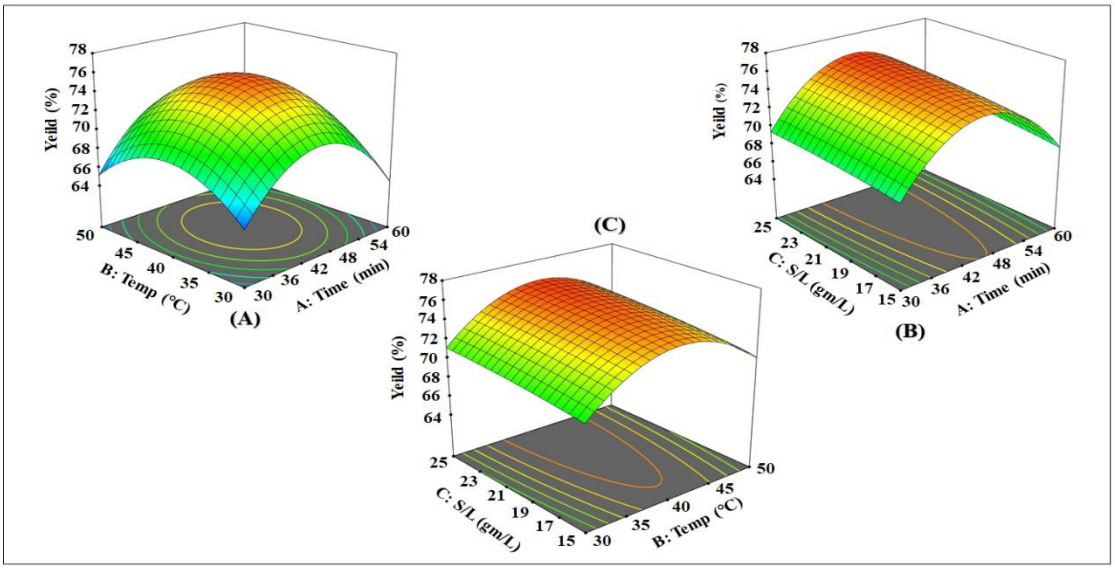

Figure 2. 3D plots representing the intersection response effects

3.3. The interaction response effects

The three-dimensional (3D) surface plots as presented in Figure 2 indicated extraction time affected, most significantly. The ASG extracts yield increased along with the increase of extraction time followed by extraction temperature effect, factor B considering that the prolonged heating resulted in a decrease in extraction yield due to the sample's thermal degradation and polymerization. The optimal conditions for ASG by UAE suggested by the model to gain high yields were specified: an extraction time of 45 min, temperature 40 °C, solid/liquid ratio of 1:25 g/ml, and the optimum yield of 75.89%. These parameters were selected for maximizing yield, with the highest overall desirability of D = 0.9. The mean values of the investigated response obtained from actual

experiments at this point were: 75.88. Figure 2 shows the interactions between the experimental levels of the tested variables and their impact on the response. You can see that the different shapes of the contour plots have different interactions between the variables. Figure 2A shows the effects of extraction time and temperature and their effect on the percentage of extraction yield. The yield of ASG extract increased as the extraction time increased, and it took a certain amount of time for the ultrasonic waves to stimulate cell wave interference before releasing the extract. A similar effect of extraction temperature on the yield of ASG extract was observed. Similar interactions between extraction temperature and solid-liquid ratio in Figure 2C were observed with ASG Ultrasonication Assisted Extraction "UAE". The results showed that increasing the extraction temperature increased the solubility of ASG and also improved the extraction yield (%). Yields on ASG extracts also increased for other reasons such as increased solvation, increased material porosity, and mass transfer. The study's results were confirmed by Maran et al. (2013).

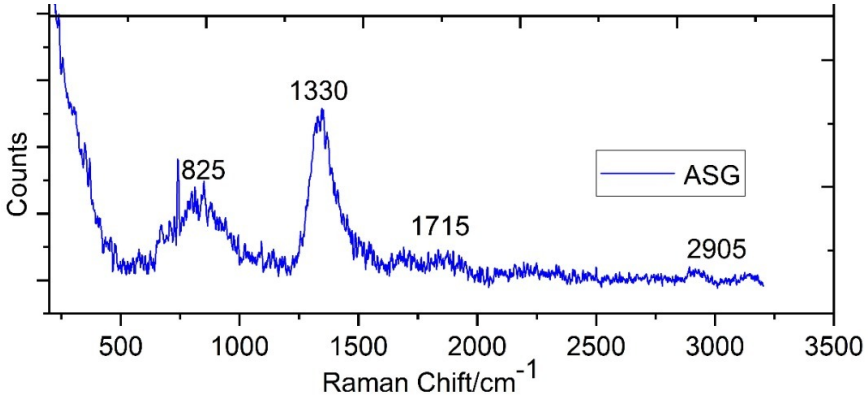

Figure 3. Typical Raman spectrum of *A. seyal* gum powder sample

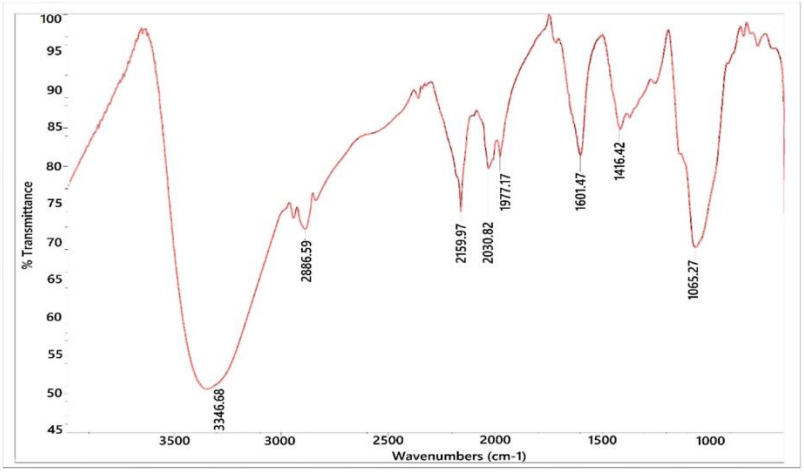

Figure 4. FTIR spectra for *A. seyal* gum powder sample

3.4. Phytoconstituent identification of ASG

The ASG Raman spectrum of ethanol extract is nearly identical to raw gum. The observed spectrum wavelength identification is shown and assigned peaks in Figure 3. The Raman spectrum of the optimized extracts revealed that the ethanol ASG extracts had three primary peaks at 1330 and 825 cm^{-1}. ASG spectra showed bands at around 1715 cm^{-1}.

The present study used FTIR analysis to recognize the functional groups of ASG powder based on their peak values. The spectroscopic characterization of the ASG sample and its components by FTIR is shown in Figure 4. The findings suggested the existence of functional groups like alkane, imine, amine, aromatic compounds or ketone, and phenol stretching. The FTIR spectrum of the ASG ethanol extract exhibited few significant differences from the native ASG FTIR spectrum. The FTIR spectrum was obtained with an ASG extract of 4000-400 cm^{-1}. The resulting spectra showed significant overlap between each component's absorption spectrum. Each band describes the absorption peaks associated with the functional groups isolated from the ASG extract. The maximum absorbance peaks of ASG were detected at 1747 cm^{-1} in the spectral band 1200-824 cm^{-1}, corresponding to the ASG carbohydrate group. Skeletal stretch vibrations of the side chains of polysaccharides create this so-called carbohydrate fingerprint area.

3.5. Cytotoxicity assay against K526 cells

Growth-inhibiting action of ASG crude ethanol extracts on K562 cells after exposure to different concentrations (10, 5, 2.5, 1.25, 0.625, 0.3125 µg/ml) suggested that the ASG treatment induced a dose-dependent inhibition of cell growth in K562 cells. The IC_{50} value for ASG in K562 cells was 10 µg/ml simultaneously. Taxol was also evaluated for its cytotoxicity resulting in a value of IC_{50}=6.3 µg/ml (Figure 5). The ASG extract concentration required to reduce cell viability by half was10 µg/ml.

The organic ASG crude extract was cytotoxic in a dose-dependent manner on two different leukemic cell lines, K562 and Jurkat T cancer cells. Cell growth inhibition was more pronounced in Jurkat cells than in the K562 cell line. These studies recommended ASG as an antitumor agent, although the mechanism of action behind it needs to be elucidated.

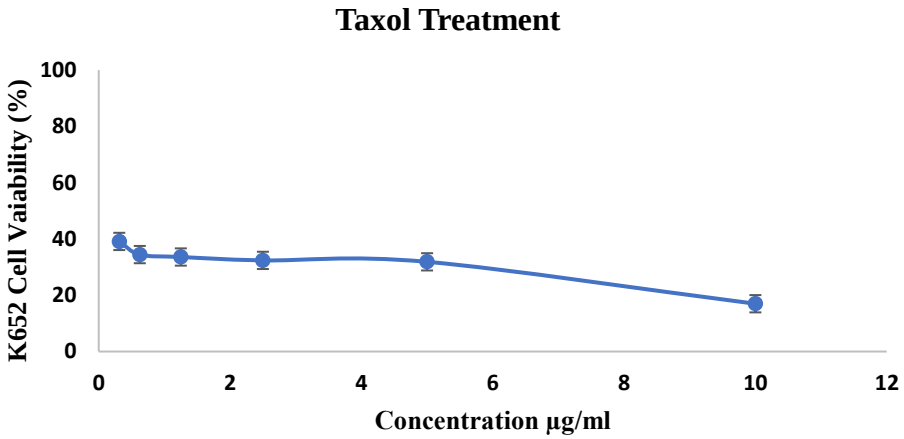

Figure 5. The cell viability percentage of K652 cell lines vs. different concentrations of Taxol treatment (R^2 = 0.9009, IC_{50}=6.3 µg/ml)

4. Conclusions

This study has proven that *Acacia* gum is an important plant genus widely used due to its ethnomedicinal therapeutic benefits as an anti-inflammatory, anti-ulcerative anticancer, immunostimulant, anti-obesity, anti-chronic renal failure, anti-diarrhea, and antitoxic effects. Treatment for leukemia can be a complex combination with many sides effects. Hence, searching for a safer and more effective treatment is considered important. This study was successfully added value to investigate the antiproliferation effect of the crude ASG extract on leukemia cancer cell lines after optimizing its metabolites yield. The ASG extraction was successfully applied to optimize the extraction conditions of gum using response surface methodology to maximize the extraction yield. Validation of the optimized condition at temperature 40 °C, with a solid-to-solvent ratio of 25 g/ml, and an extraction time of 45 min exhibited an extraction yield of 75.89% ± 0.52. Moreover, comparing the UAE extraction method with conventional extraction methods proved to be more efficient regarding extraction efficiency, shorter extraction time, and the small quantity of liquid used, making it environmentally friendly.

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Conflict of interest

The authors confirm that there are no known conflicts of interest.

CRediT authorship contribution statement

Nassereldeen Kabbashi: Conceptualization, Investigation, Data analysis, Supervision
Tahani Maher Alawdat: Writing-original draft; Resources, Conceptualization, Visualization, Formal analysis, Investigation, Methodology
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Supplementary File

None.

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